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Circadian Control of Insulin Secretion Is Independent of the Temporal Distribution of Feeding

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KALSBEEK, A. AND J. H. STRUBBE. *Circadian control of insulin secretion is independent of the temporal distribution of feeding.* *PHYSIOL BEHAV* 63(4) 553–558, 1998.—To investigate whether there is a circadian regulation of insulin secretion, rats were adapted to a feeding regimen of six meals equally distributed over 24 h. Under these conditions basal glucose and insulin levels increased during the light phase and decreased during the dark phase. Maximal blood glucose responses were fairly similar during the six different meals, but glucose increments were clearly delayed during the last two meals consumed during the light period. Insulin increments were highest during the dark phase and clearly diminished during the second half of the light phase. This situation was reversed when the scheduled meals were replaced by i.v. glucose infusions, i.e., no significant differences were detected between insulin responses, whereas glucose increments were reduced during the dark period. These results show that there is a circadian regulation of basal blood glucose and feeding-induced insulin responses, which is *independent* of the temporal distribution of feeding activity. © 1998 Elsevier Science Inc.

Plasma glucose Food intake Pancreas Suprachiasmatic nucleus

THE main physiological stimulus for insulin secretion in humans and animals is food intake, but the size of the insulin response after food intake may also depend on the time of day (1,11,20,28,32,39,46). However, in all of these previous studies the fasting period preceding a meal was different for morning and evening meals. Because it is known that even short-term fasts may influence insulin responses (44,52), it is not clear whether the differences observed in previous studies are due to the unequal distribution of feeding activity or reflect a true circadian modulation. Most if not all daily rhythms are driven by the circadian oscillator that is contained in the mammalian suprachiasmatic nucleus (SCN). Indeed, there is ample evidence of the involvement of the SCN in the control of behavioral rhythms such as locomotor activity, sleep-wake and feeding behavior (37). Also the involvement of the SCN in the circadian control of anterior pituitary hormonal release has been clearly established (22,23,50). However, the direct role of the SCN in the regulation of autonomic nervous activity and more peripheral hormonal systems is less obvious. Daily variations in these parameters often are a direct consequence of ongoing behavioral rhythms. For instance, daily variations in blood pressure, heart rate and body temperature heavily depend on the amount of activity (25,36). The same may hold for the activity of a peripheral hormonal system such as the endocrine pancreas. The rhythm of insulin release seems to be mainly driven by a

rise in blood glucose levels as a consequence of a circadian pattern in feeding behavior.

One possible research strategy to delineate the impact of the circadian oscillator on autonomic processes and peripheral hormonal systems is to study these parameters without the interference from behavior. In this way circadian rhythms in blood pressure and body temperature have been revealed in humans during continuous bed rest (34,53). Similarly, persistent rhythms in plasma glucose and insulin have been reported in the absence of feeding behavior (i.e., fasting), in both human (15) and animal studies (2,26). Yet another approach to create a constant environment is to present the disruptive (behavioral) stimulus constantly or at regular intervals. In this way too circadian rhythms in plasma glucose and insulin have been described (5,47–49,52), though only in human studies. However, rhythms associated with feeding, such as intestinal glucose absorption, may persist for several days, even without food intake (38). Therefore, short-term studies on the diurnal variations in i.v. or oral glucose tolerance may still experience interference from the previous feeding history, notwithstanding a similar duration of the earlier period of fasting. After SCN lesions glucose and insulin responses are similar during day and night, but the distribution of the meals across the light/dark cycle is also equal now (45). Therefore, it might still be so that the distribution of feeding activity is the primary determinant of daily

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variations in insulin and glucose responses, and not the circadian oscillator located in the SCN.

The experiments in the present study were therefore designed to unmask a possible direct control of the endogenous pacemaker on the circadian aspects of insulin release by the endocrine pancreas. To exclude daily variations in the amount of food consumed or the length of the earlier period of fasting, we subjected rats to a feeding regimen of six meals a day, spaced 4 h apart. After at least 2 weeks of feeding on this regimen (thus avoiding any acute effects of the induced experimental protocol) the impact of the circadian oscillator on pancreatic insulin release was measured by determining the plasma profiles of glucose and insulin during each meal.

MATERIALS AND METHODS

Animals and Food Intake

Male Wistar rats weighing 320–350 g were kept individually in perspex cages (25 × 25 × 30 cm) in a sound-attenuated room on a 12 h light:12 h dark regime (lights on at 0700 hours) at an ambient room temperature of 20°C. Food pellets (2–3 g) of lab chow (Murakon, Trouw, The Netherlands) were available in metal food hoppers. A rat could gnaw off pieces of food through vertical stainless steel bars situated at the front of the food hopper. Spillage was collected in an undertray attached to the food hopper. The hopper was weighed continuously throughout the day-night cycle so that the size of every individual meal was obtained. Access to food could be prevented by a sliding door situated in front of the food hopper. Door opening and door closing were activated by air pressure and controlled by a clock. Water was available ad lib. All of the following animal experiments were conducted under the approval of the Animal Care Committee.

Blood Sampling

All rats were provided with a permanent heart catheter that allowed blood sampling in the undisturbed rat (41). Before the onset of the experiments the rats were handled for at least 1 week to accustom them to the blood sampling procedure. Blood samples of 0.3 mL were taken and heparinized. During a meal, blood samples were taken at –30, –10, –5 and 0 (immediately before the opening of the doors) and 1, 3, 5, 10, 15, 25, 35, 45 and 60 min after the start of food availability. Blood was replaced during the experiments by transfusion of heparinized blood (25 U/mL) of a donor rat fed ad lib. Transfusions of 0.3 mL were given in the sampling intervals of 5 min and longer (i.e., ± 1.2 mL of blood was not replaced). Previous reports have shown that this amount of blood can be removed with no risk of stimulating the hypothalamo-pituitary-adrenal axis (10,23). In addition, normal blood composition (such as the hematocrit value) is not affected by this sampling protocol (3,27,54).

Experimental Procedure and Design

The rats were entrained to a feeding schedule of six 10-min meals spaced equally over the day-night cycle. Food became available at Zeitgeber Time (ZT) 2, ZT6, ZT10, ZT14, ZT18, and ZT22 (ZT12 being defined as the onset of the dark period). Animals adapted to the feeding schedule in about 2 weeks. Adaptation was considered completed when animals had learned to consume ± 3 g during every meal. The glucose and insulin responses as a result of ingestion of these meals were sampled in random order. Animals were used for a blood sampling experiment only once every 4 days, with only 1 ZT point being sampled per animal on a particular day. Sampling sessions were performed in Weeks 3 to 8 after the start of the six meal per day schedule.

Intravenous glucose infusions were administered in randomized order during the six periods when food intake normally occurred. The infusion of 0.1 mL/min of glucose, either 10% or 7% (W/V), was postponed until 15 min after the ordinary meal onset (no food was offered) to avoid conditioning influences. The rats were allowed to eat 3.3 g after the i.v. infusion to prevent an eventual caloric deficit. During glucose infusions blood samples were taken at –10, 0, 1, 2, 3, 5, 10, 15, 20, 25, 35, and 45 min after the start of the glucose infusions. Sampling sessions were organized in the same way as those during meal-feeding.

Insulin and Glucose Determination

Blood samples were immediately chilled at 0°C, and the blood glucose concentration was measured in 50 μ L of whole blood by a ferricyanide method with a Technicon analyser. The remaining blood was centrifuged at 4°C and the plasma was stored at –20°C until analysis. Plasma insulin was measured by radio immunoassay (NOVO-Denmark) using rat insulin as a standard, 125 I-labeled porcine insulin, and anti-porcine insulin guinea pig serum M-8309. Samples (25 μ L) were measured in duplicate. Bound and free 125 I-labeled insulin were separated from each other by polyethylene glycol (23.75% wt/wt in water) precipitation. The coefficient of variation of the immunoassay was <8%.

Statistical Evaluation

The results are expressed as means \pm SEM. Statistical analysis was conducted using MANOVA and the student Newman-Keuls test as a post-hoc analysis. Paired Student's *t*-tests were used to detect significant differences from basal (i.e., $t = 0$) values. $p < 0.05$ (with the Bonferroni correction when necessary) was considered a significant difference.

RESULTS

Feeding activity was equally distributed over the light/dark cycle, restricted to the six periods of door opening. Despite the equally distributed feeding activity, general (locomotor) activity still showed a clear day-night rhythm (Fig. 1), with the major part of activity occurring during the dark period. Animals adapted readily to the aberrant feeding schedule, and resumed their normal growth rate (i.e., ± 3.5 g/day) within 1 week. Rapid adaptation probably is possible because meal size and the number of meals deviate only slightly from those in ad lib conditions, in contrast to the more often used one or two meal per day schedules. These schedules force the animals to eat 8–16 g/meal, asking for considerable metabolic adaptations. The major difference between our six meal per day schedule and the more demanding one or two meal per day feeding schedule is also indicated by the absence of anticipatory locomotor activity (Fig. 1) and a conditioned insulin response (43). Table 1 shows the mean size of the six meals during the Week 4 of the experiment (A), and the actual amount of food consumed during the different blood sampling sessions (B). Considering all the meals consumed during one week (i.e., 7 meals/ZT/animal) shows that mean night time meals are slightly larger than day time meals. However, when only the meal size during the actual blood sampling sessions is considered no significant differences were detected.

Mean basal (i.e., $t = 0$) glucose and insulin levels just before the six meals varied along the light/dark cycle (Fig. 2; absolute values are shown in Table 2). Both plasma glucose and insulin levels steadily increased from the onset of the light period, reaching peak levels just before (insulin) or after (glucose) the onset of the dark period. Only the glucose variations, however, reached statistical significance (glucose: $F(5) = 2.45$, $p = 0.039$; insulin:

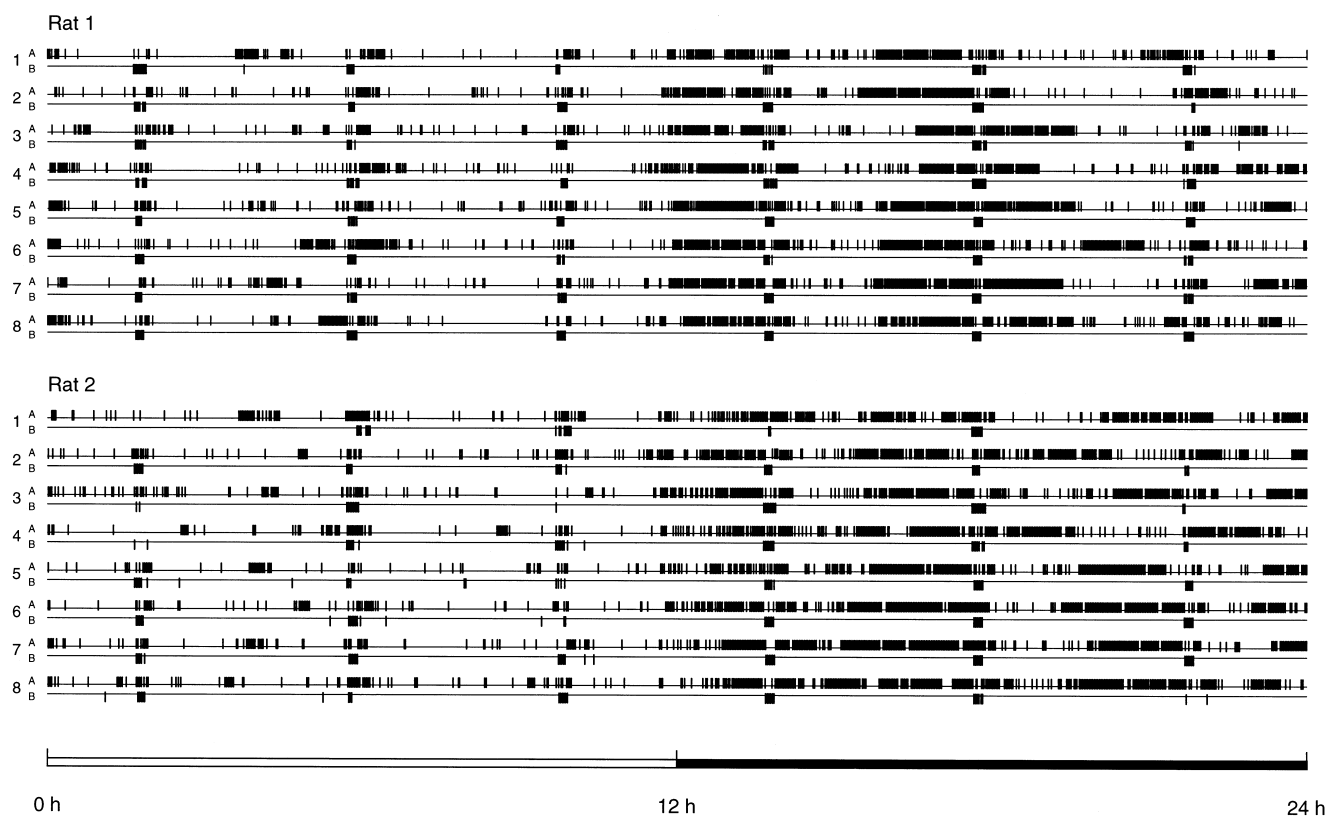


FIG. 1. Locomotor and feeding activity of two rats during 8 days on the scheduled feeding regime. In both animals feeding activity (B) is equally distributed over the light/dark cycle, whereas locomotor activity (A) is still for the largest part nocturnal.

$F(5) = 1.12$, $p = 0.354$). Post-hoc analysis showed that peak glucose values at ZT14 were significantly higher than those at ZT2 and ZT22.

Feeding

Feeding-evoked increases in plasma glucose and insulin release at the different ZTs are displayed in Fig. 3. To circumvent the aforementioned significant daily variations in basal levels, responses are expressed as the difference compared with the respective $t = 0$ values. MANOVA comparison of the six different glucose increments indicated a highly significant effect of *Feeding* ($F(9, 288) = 45.86$, $p < 0.001$), no significant effect of *ZT* ($F(5, 32) = 2.03$, $p = 0.10$), but the *interaction* effect again was clearly significant ($F(45, 288) = 1.52$, $p = 0.02$). This analysis indicates that every meal induced a significant increase of plasma glucose,

but also that the feeding induced glucose increments at the various ZTs differed. During every meal peak levels were reached 10–15 min after door opening. However, during certain meals the rise in plasma glucose was clearly delayed (i.e., especially at ZT6 and ZT10), resulting in the clear *interaction* effect detected by ANOVA. Regression analysis showed no significant covariance between meal size and maximal glucose responses ($p = 0.69$).

During all six meals mean plasma insulin levels rose within the first minute after food access. However, at ZT6 and ZT10 these increases were not significant. Meals consumed during the dark phase or shortly after onset of the light phase showed further increments of plasma insulin in the subsequent minutes (i.e., 3–10 min after door opening), but at ZT6 and ZT10 significance was only reached at $t = 15$. Highest mean levels of plasma insulin levels were attained 15–30 min after opening of the door.

TABLE 1
MEAN MEAL SIZE DURING THE SIX SCHEDULED FEEDING OPPORTUNITIES

ZT2	ZT6	ZT10	ZT14	ZT18	ZT22
A) Weekly mean					
$3.1 \pm 0.1^*$	3.0 ± 0.2	2.8 ± 0.3	3.4 ± 0.1	3.4 ± 0.2	3.3 ± 0.3
B) Mean during blood sampling					
$3.3 \pm 0.3^\ddagger$	2.5 ± 0.3	3.1 ± 0.3	2.9 ± 0.2	3.5 ± 0.2	3.0 ± 0.4

* $n = 40$ –50; $^\ddagger n = 6$ –8

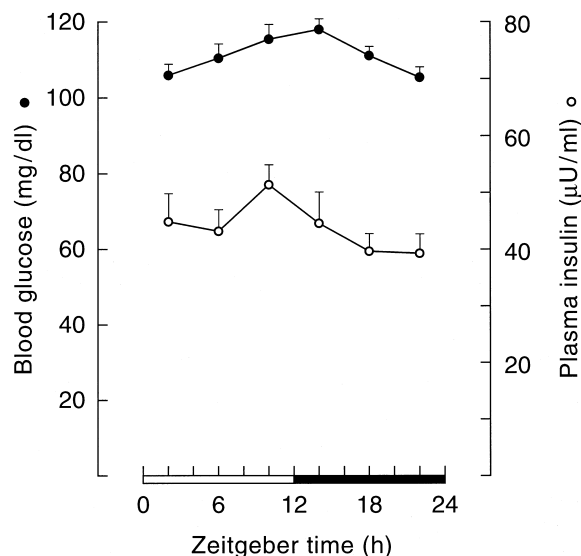


FIG. 2. Basal (i.e., $t = 0$) plasma insulin (\circ — \circ) and blood glucose (\bullet — \bullet) levels across the day-night cycle. Values are means \pm SEM from the three experiments described in the present study, i.e., feeding and 10% and 7% glucose infusions ($n = 15$ – 18). Meals were initiated at ZT2, 6, 10, 14, 18, and 22. The black area indicates night.

MANOVA detected significant effects of *Feeding* ($F(9, 306) = 18.32, p < 0.001$), *ZT* ($F(5, 34) = 4.49, p = 0.003$), and the *interaction* between those two factors ($F(45, 306) = 1.45, p = 0.039$), indicating significant differences between the feeding-induced insulin increments depending on the time of the day. Post-hoc analysis revealed the most significant differences between meals eaten at ZT6 and ZT10 on the one hand and meals eaten at ZT2, ZT14, ZT18, and ZT22 on the other. Regression analysis did not indicate a significant covariation of meal size and maximal insulin increments ($p = 0.35$).

Glucose Infusions

Infusion of a 10% glucose solution caused immediate and pronounced increases of both plasma glucose and insulin (45–60 mg% and 80–120 μ U/mL, respectively) that were much higher than those induced by ingestion of a meal (compare with Fig. 3).

Therefore, a new experiment was started using a 7% glucose solution. With this glucose concentration maximal insulin responses were comparable with those elicited by the consumption of a meal (i.e., 60–80 μ U/mL).

Starting immediately after the start of the infusion of the 7% glucose solution, blood glucose levels increased, the most rapid rise occurring during the initial 5 min of the infusion period (Fig. 4). Blood glucose levels continued to rise until the infusions were discontinued at $t = 15$ min. Blood glucose then rapidly decreased, preinfusion levels being reached 20 min after the end of the infusion. After stopping the infusion, blood glucose levels dropped very rapidly and the concentration even dropped below basal levels during most of the infusions. MANOVA revealed significant effects of *infusion* ($F(8, 216) = 336.7, p < 0.001$), of *ZT* ($F(5, 27) = 2.67, p = 0.04$) and *interaction* ($F(40, 216) = 3.68, p < 0.001$), thus indicating that the infusion-induced glucose increments differ depending on the time of day. Indeed, post-hoc analysis revealed that the glucose response in the middle of the night (ZT18) was lower than the responses at ZT6, 10 and 14.

Parallel with the rapid rise of blood glucose, plasma insulin markedly increased from $t = 1$ onwards. In fact, the $t = 1$ values marked the initial peak of a typical biphasic pattern of response. A secondary rise of plasma insulin occurred from 5 min onward and lasted until the glucose infusion was terminated at $t = 15$ min. Plasma insulin then rapidly abated to baseline values. MANOVA again detected a significant effect of *infusion* ($F(8, 216) = 124.72, p < 0.001$), whereas the effect of *ZT* just escaped significance ($F(5, 27) = 2.21, p = 0.08$) and also the *interaction* effect did not reach significance ($F(40, 216) = 1.32, p = 0.11$), indicating fairly similar insulin responses during every glucose infusion.

Insulinogenic Index

The sensitivity of feeding-induced insulin responses, expressed as the insulinogenic index (quotient of insulin and glucose increment) at $t = 15$, varied considerably along the light/dark cycle (Fig. 5). ANOVA, however, showed no significant effect of *ZT* ($F(5) = 1.831, p = 0.135$). The insulinogenic index for both the 7% and 10% glucose infusion was very similar across the light/dark cycle (Fig. 5). This was substantiated by the statistical analysis, showing no effect of *ZT* on either condition (7%, $F(5) = 1.296, p = 0.256$; 10%, $F(5) = 0.724, p = 0.615$). However, overall analysis of the three test conditions (i.e., feeding, 7% or 10% glucose infusion) showed a very significant effect of *treatment* ($F(2, 13) = 14.39, p < 0.001$), whereas no effects of *ZT* or *interaction* were detected ($p > 0.1$). Post-hoc analysis revealed

TABLE 2
ABSOLUTE PLASMA GLUCOSE AND INSULIN LEVELS AT $T = 0$ DURING THE SIX SCHEDULED FEEDING OPPORTUNITIES ($N = 5$ – 8)

	ZT2	ZT6	ZT10	ZT14	ZT18	ZT22
A) Feeding						
Glucose						
98.7 ± 3.0	96.9 ± 3.3	103.0 ± 1.8	109.4 ± 2.5	105.6 ± 2.7	102.5 ± 1.5	
Insulin						
29.8 ± 3.7	33.8 ± 5.3	46.8 ± 7.9	32.6 ± 3.1	30.3 ± 2.8	38.5 ± 6.9	
B) 7%-glucose						
Glucose						
117.2 ± 3.4	130.8 ± 2.6	132.5 ± 2.2	130.3 ± 1.4	119.2 ± 3.0	111.2 ± 6.4	
Insulin						
63.0 ± 6.1	49.7 ± 8.3	51.0 ± 3.4	39.7 ± 6.1	46.2 ± 6.8	38.8 ± 3.9	

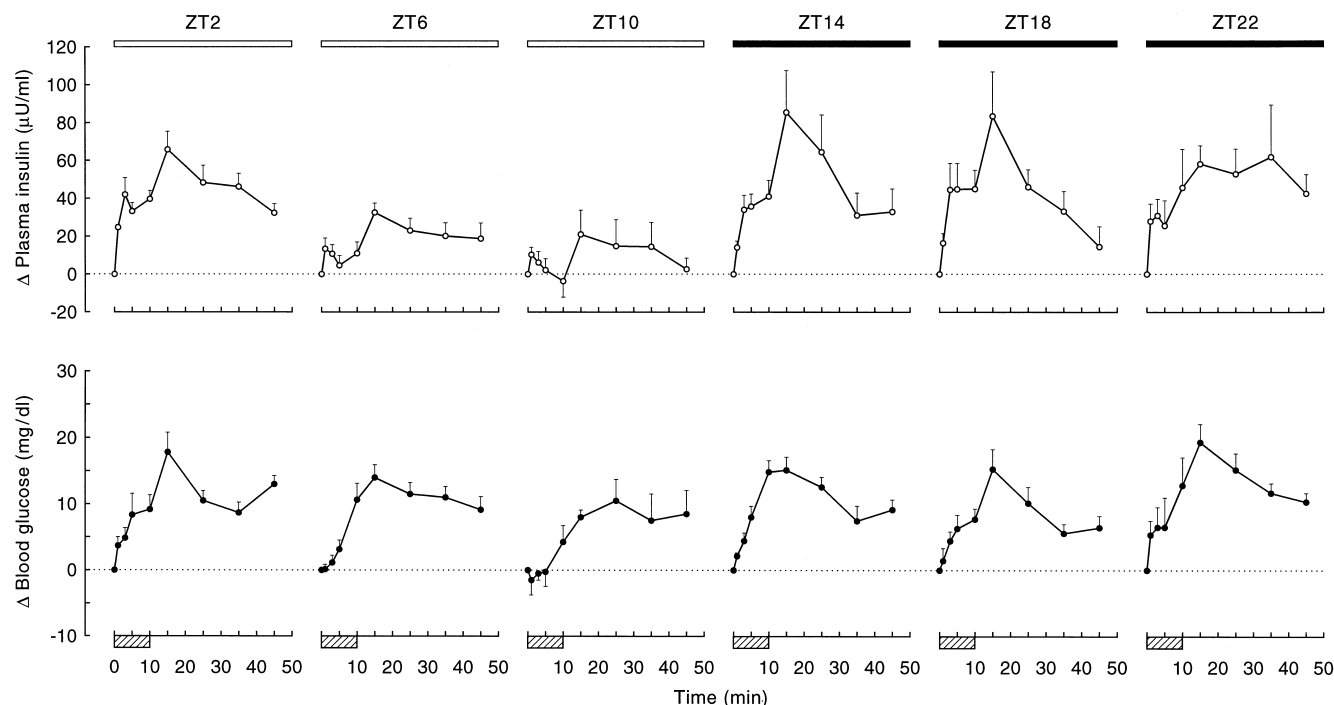


FIG. 3. Plasma insulin and blood glucose responses ($n = 6-8$) after meal ingestion during day and night time on a scheduled feeding regimen. Boxes indicate meals. The black area indicates night. Responses are expressed as the difference compared with the respective $t = 0$ values. Absolute values at $t = 0$ are displayed in Table 2.

that the insulinogenic index for feeding was higher than those for glucose infusions at ZT14, ZT18 and ZT22.

DISCUSSION

The results of the present study provide clear evidence for a direct control of the endogenous circadian pacemaker on both basal blood glucose and feeding-induced blood glucose and plasma insulin responses. Provided meal size, intake rate, and prior fasting period are all kept constant, basal levels of plasma glucose show significant fluctuations along the light/dark cycle. Although there was a similar tendency for basal insulin levels, these fluctuations did not reach statistical significance. Under "normal" ad lib conditions circadian influences on basal levels of plasma glucose and insulin cannot be assessed, since they are strongly influenced by prevailing feeding conditions. Therefore, the first indications for a circadian regulation of basal glucose and insulin levels independent of food intake, came from studies in which subjects had been fasting (2,15,26), although the results were not equivocal. More recently in humans the "dawn-phenomenon," i.e., higher glucose output and insulin requirements during the early morning hours, was described in a few studies (7,8), indicating a circadian regulation of basal glucose levels. This "dawn-phenomenon" very much resembles the pattern of plasma glucose and insulin levels observed in the present study, when taking into account the 12-h shift due to the diurnal (human) or nocturnal (rat) activity pattern. In both instances the peak in plasma glucose levels is closely related to the circadian peak in circulating plasma glucocorticoids (cortisol or corticosterone) shortly before the onset of the main activity period. Initially the "dawn-phenomenon" was ascribed to the hyperglycaemic action of this early morning cortisol peak (6,49), since plasma glucagon levels are not increased. However, suppression of the cortisol rise could not prevent the "dawn-

phenomenon" (8). Therefore, an important role has been attributed to the nocturnal surges in growth hormone (6,40,49). More recent evidence, however, suggests that an increased hepatic glucose production is the main factor responsible for the "dawn-phenomenon" (4).

Next to the circadian control over the basal (unstimulated) levels of blood glucose, and insulin and glucose increments, as a consequence of feeding activity, clearly differed depending on the time of day, confirming numerous previous observations in rat and human (1,11,19,20,28,32,39,46,48,52). However, the present study is the first to show unequivocally that the variation in these responses along the 24-h cycle is independent of an unequal distribution of feeding activity. Insulin responses were most profoundly affected (i.e., decreased) during the second half of the light period (ZT6 and ZT10), and glucose increments were clearly delayed during the first 5 min of these two meals. Therefore, a significant daily variation was present despite the fact that feeding conditions, such as prior fasting period, amount of food intake and intake rate, were very much similar for all six meals. These results indicate that the most prominent (inhibitory) effects of the circadian timing system on feeding-induced insulin release are present at the end of the rest period. Human studies, in general, show diminished insulin responses as a consequence of meals consumed before or at the start of the activity period [i.e., breakfast; (1,11,39,52)], and thus show similarity to our animal data. However, since a number of studies restricted their meals to the normal human activity period (i.e., 0700 to 1900 hours), considering only one-half of the 24-h cycle, differences may be small and even reversed (20,28,48,52). The present study clearly shows that the most pronounced attenuation of insulin responses is not yet apparent at the beginning of the sleep period, and only develops during the second half of the sleep period. In the morning, however, this

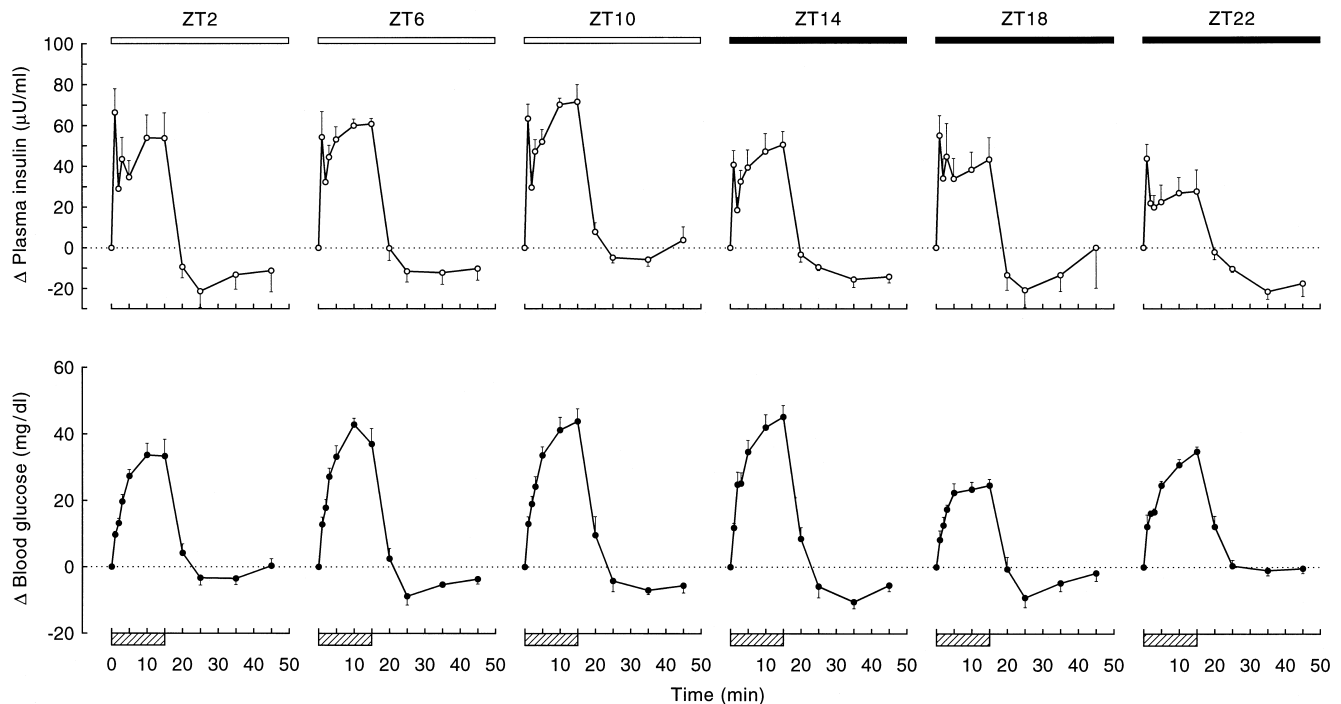


FIG. 4. Plasma insulin and blood glucose responses ($n = 5-6$) after intravenous glucose infusion (7%) during day and night on a scheduled feeding regime. Boxes indicate the time of infusion. The black area indicates night. Responses are expressed as the difference compared with the respective $t = 0$ values. Absolute values at $t = 0$ are displayed in Table 2.

inhibition is rapidly reversed into a stimulation of insulin release shortly after awakening. Recently, Boden et al. (5) found a clear circadian rhythm of insulin secretion in humans when plasma glucose was clamped at different levels of glycemia. Minimal insulin secretion rates were found at the end of the sleep period.

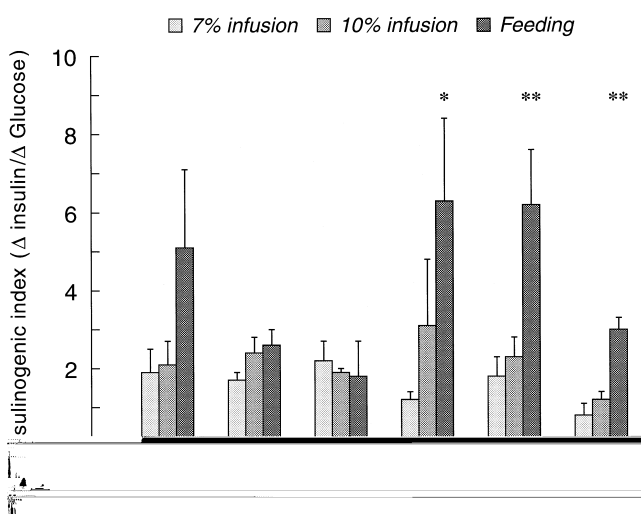


FIG. 5. Insulinogenic index (i.e., insulin response/glucose response) at $t = 15$ min after intravenous glucose infusions and meal taking (mean \pm SEM). The black area indicates night. Asterisks indicate a significantly higher index for the feeding response as compared with only the 7% glucose infusion (*) or both the 7% and 10% infusions (**).

Indeed, their plots of the circadian rhythm of insulin secretion perfectly match the rhythm of maximal feeding-induced insulin responses as we observed.

The feeding data show a pronounced delay in the rate of glucose entrance in the general circulation during feeding at ZT6 and ZT10, suggesting a reduced intestinal glucose absorption. Indeed, it has been reported that glucose absorption is high at the beginning of the dark phase and low during the light phase (13,17,42), and the same holds for the activity of the intestinal enzymes maltase and sucrase (42). Also stomach emptying is highest in the beginning of the dark period (31). In addition, however, the higher basal plasma insulin levels at this time of the day may add to the delay in glucose rise. However, the delayed entrance of glucose in the circulation will mainly affect the first phase of insulin release (1–5 min). Additional factors may add to the strong attenuation of the second phase of insulin release (5–20 min). For instance, during feeding an important part of the insulin secretion is evoked by the release of gut factors (i.e., incretins) such as glucagon-like peptide-1 (GLP-1) (12,18). The potentiating effect of the gut factors is clearly indicated in the present study by the higher insulinogenic index for feeding, indicating that glucose from oral routes is much more effective in stimulating insulin release than when infused i.v. The circadian regulation of this feeding-induced release of gastrointestinal peptides is indicated by the fact that the insulinogenic index for feeding was significantly higher than those for glucose infusions during the dark period, but not during the light period. In fact, at ZT6 and ZT10 the mean insulinogenic index for feeding and glucose infusions was identical, indicating a complete absence of additional (gastrointestinal) factors stimulating the release of insulin at this time of the day. Together, these data show that the reduced meal-induced insulin responses at ZT6 and ZT10 are caused by a combination of factors,

the most important being a delayed intestinal glucose absorption, higher basal plasma insulin levels, and an absence of insulinotropic gut factors.

In contrast to the marked diurnal differences elicited by the subsequent feeding periods, insulin increments caused by the infusion of glucose in the general circulation were fairly comparable across the light/dark cycle. The infusion data, therefore, do not indicate an important role for the circadian timing system on the glucose stimulated pancreatic release of insulin *per se*. A number of human studies have reported increased glucose and insulin levels during the night (14,33,47,51) under conditions of a constant glucose infusion. This nocturnal increase of plasma glucose has been related to a decrease in glucose utilization by peripheral tissues (i.e., brain and muscles) during sleep. Therefore, these infusion studies seem to indicate primarily a daily rhythm in glucose utilization (24), and not in insulin secretion. Indeed, when glucose levels are clamped, the highest insulin secretion rates are found during daytime again (5). In our scheduled feeding condition too, infusion of a glucose solution resulted in the highest blood glucose levels during the main sleep period of the rat. Insulin levels showed a similar trend. The delayed intestinal glucose absorption and higher basal plasma insulin levels with meal feeding at ZT6 and ZT10 finally resulted in only a small reduction of the maximal glucose increments, due to the reduced peripheral glucose utilization at this time of the day. In addition, the rhythm in glucose utilization may add to the "dawn-phenomenon" and the circadian variation in basal glucose levels we observed, since the lower glucose utilization at the end of the main sleeping period will tend to increase basal blood glucose levels.

In conclusion, when rats are accustomed to eating six identical meals equally distributed over the light/dark cycle, basal blood glucose levels and feeding-induced insulin responses still show significant daily variations. Because these results are in line with previous results obtained under free-feeding conditions, this indi-

cates a circadian control of blood glucose levels and insulin responses *independent* of food intake. The present experiments did not test directly the involvement of the autonomic system in the circadian modulation of glucose and insulin responses, nevertheless we feel previous experiments of ourselves and others provide indications to suspect that an important aspect of the circadian regulation of glucose homeostasis involves SCN control over the sympathovagal balance of the autonomic system. For instance, peak levels in basal blood glucose at the transition from the light to the dark period probably are caused by a decreased peripheral glucose utilization and increased hepatic glucose production, as a consequence of an increased activation of the sympathetic system (8). Indeed, electrical stimulation of the SCN causes hyperglycemia, an effect which can be prevented by the administration of α - and β -adrenergic blockers (16,30). Furthermore, experiments aimed at elucidating the circadian regulation of the daily corticosterone peak have provided clear evidence for an SCN control via the sympathetic innervation of the adrenal gland (9,21–23). On the other hand, our previous experiment (45) shows that the pronounced daily rhythmicity in insulin responses is most likely effectuated via an inhibitory action of the SCN on the parasympathetic input to the different levels of the gastrointestinal system (i.e., stomach, intestine, pancreas, liver) during the second half of the light period (29,31,35,45). Follow-up experiments are aimed at investigating further the respective roles of counter-regulatory hormones and the autonomic nervous system in the presently described circadian variations in glucose and insulin responses.

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